## Assimilation in Bean Plants of Nitrogen from Saline Solutions<sup>1</sup>

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T WAS pointed out in a previous paper (3) that increasing concentrations of either NaCl or CaCl<sub>2</sub> in the substrate influenced the percentage composition of nitrogen as well as of other nutritional elements in bean plants. Since plant performance is dependent upon the synthesis of inorganic nitrogen into the proteinaceous materials of active protoplasm, it became desirable to know to what extent modifications in plant response were associated with modifications in certain phases of nitrogen assimilation. Since nitrogen metabolism is conditioned by the status of carbohydrates in the plant, it was considered essential to carry out the investigation with plants grown at a season of the year when sunlight is at a minimum as well as when it is at the maximum. This report deals with plants grown under the former conditions.

The experimental technique by which these red kidney bean plants were grown has been previously described (3). Upon harvesting, the plant tissue was prepared for analyses by desiccation in a rapid current of air 70 degrees C. Nitrogen partition was carried out according to the technique described by Clark (1). Sugars were analyzed as per Tompsett's (5) procedure. Sucrose was inverted with invertase and starch was hydrolized with fresh salivary diastase.

The growth performance of these plants is presented in Table I.

TABLE I-AVERAGE GREEN WEIGHTS, TOP/ROOT RATIOS, AND DRY MATTER AS PERCENTAGE OF GREEN WEIGHT OF BEAN PLANTS AS INFLUENCED BY INCREMENTS OF NACL AND CACL<sub>2</sub> IN THE SUBSTRATE

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	Base Nutri- ent (Gms)	NaCl Se	NaCl Series (Gms)				CaCl <sub>2</sub> Series (Gms)				
Osmotic Concention	0.5 1.	5 2.5	3.5	4.5	1.5	2.5	3.5	4.5			
Green Weights											
Blades	51.6 22.2 73.8	$ \begin{array}{c cccc} 30.5 & 24.1 \\ 14.9 & 12.2 \\ 45.4 & 36.3 \\ 21.3 & 18.5 \\ 66.7 & 54.8 \\ 2.1 & 2.0 \\ \end{array} $	29.6 14.2 43.8	$\begin{array}{c c} 13.8 \\ 6.2 \\ 20.0 \\ 10.8 \\ 30.8 \\ 1.9 \end{array}$	28.6 13.6 42.2 16.2 58.4 2.6	26.1 11.2 37.3 13.9 51.2 2.7	16.9 7.4 24.3 9.1 33.4 2.7	14.0 5.7 19.7 8.1 27.8 2.4			
		Dry Matter as	Per Cent	of Green V	Veight						
Blades. Stem + petioles. Tops. ROOI. Whole plant	10.94 8.85 10.23 5.45 8.79	$ \begin{vmatrix} 10.62 & 10.8 \\ 9.34 & 9.8 \\ 10.19 & 10.5 \\ 5.32 & 5.2 \\ 8.63 & 8.7 \end{vmatrix} $	$egin{array}{c c} 7 & 10.44 \\ 2 & 10.20 \\ 4 & 5.20 \\ \hline \end{array}$	11.05 11.00 5.32	10.96 9.93 10.62 6.16 9.38	10.43 10.38 6.66	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	11.62 11.37 6.71			

<sup>&#</sup>x27;Contribution from the United States Regional Salinity Laboratory, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the eleven western states, and the Territory of Hawaii.

Growth reductions for the whole plants were practically linear with increments in osmotic concentration of the added salt. The green weights of the calcium chloride series of plants (tops and roots) was constantly lower than those of the sodium chloride series. Yet for a given osmotic concentration there appeared to be no significant difference in the green weight of the tops of the two series of plants at respective osmotic concentrations. The differences in weight between parallel observations of the two series for whole plants was due mainly to a consistently large differential in the weights of roots. There were no visual differences in the appearance and quality of growth between the calcium and sodium plants at equal osmotic concentrations. With increasing salt concentration there was a tendency in both series for the leaves to become smaller and to have a deeper green color. These characteristics are similar to those usually associated with xeromorphism,

The roots in the calcium chloride series were consistently smaller than those in the sodium series and this is further reflected in the relatively high top-root ratios for the calcium series.

The percentage dry matter in the leaves of both series of plants showed little consistent trend with treatment. Leaves from some salt treatments were found to be more succulent than those from the base nutrient treatment. In contrast, the stems and petioles showed consistent increases in percentage dry matter with increase in osmotic concentration of the substrate-the calcium chloride series of plants having higher percentages of dry matter than the sodium chloride series. In view of the fact that an increase in osmotic concentration of the substrate restricts water absorption (2, 4) such a trend would be expected. Such small and negligible changes as have been noted in the percentage dry matter of these plants within a series are unquestionably contingent upon the fact that the experiment was carried out in December when temperatures in the experimental greenhouse were relatively moderate, and normal light was at the annual minimum. However, the data is pertinent to field conditions because production of most crops in saline regions of the southwest is most successful during the winter months.

It is striking that the roots of the plants in the calcium chloride series had a consistently higher percentage of dry matter than those of the sodium chloride series, although there was little change with osmotic concentration within a series. It was pointed out previously (3) that most of the sodium absorbed by the plants in the sodium chloride series was localized in the roots. It is possible that the presence of appreciable amounts of sodium in these roots as contrasted with accumulations of calcium in the roots of the other series has accounted for a higher degree of hydration of the protoplasmic constituents. Such a condition would be in complete agreement with the usually observed effect of sodium and calcium upon colloidal systems.

The composition of these plants with respect to certain biochemical constituents is shown in Table II. A study of the inorganic constituents of these plants brought out the fact that increasing increments of salts in the substrate caused a marked decrease in the

TABLE II—Nitrogen and Carbohydrate Fractions of Bean Plants as Influenced by Various Concentration of Added Salts in the Nutrient Solution (Green Weight Basis)

	Base Nutrient (Per Cent)	rient NaCl Series (Per Cent)			CaCl. Series (Per Cent)					
Osmotic Concentration	0.5	1.5	2.5	3.5	4.5	1.5	2.5	3.5	4.5	
Leaves										
Total assimilated N Protein N Soluble organic N Nitrate N Reducing sugars Sucrose Total sugars Starch + dextrins Total carbohydrate	0.488 0.392 0.096 0.079 0.066 0.142 0.207 0.389 0.597	$\begin{array}{c} 0.470 \\ 0.361 \\ 0.109 \\ 0.051 \\ 0.091 \\ 0.217 \\ 0.308 \\ 0.296 \\ 0.604 \end{array}$	$\begin{array}{c} 0.466 \\ 0.360 \\ 0.105 \\ 0.039 \\ 0.099 \\ 0.241 \\ 0.340 \\ 0.350 \\ 0.690 \end{array}$	$ \begin{array}{c} 0.427 \\ 0.326 \\ 0.101 \\ 0.037 \\ 0.111 \\ 0.223 \\ 0.334 \\ 0.385 \\ 0.719 \\ \end{array} $	$ \begin{array}{c} 0.436 \\ 0.335 \\ 0.101 \\ 0.024 \\ 0.128 \\ 0.242 \\ 0.371 \\ 0.375 \\ 0.745 \end{array} $	0.458 0.373 0.090 0.048 0.094 0.296 0.390 0.274 0.664	$ \begin{array}{c} 0.439 \\ 0.346 \\ 0.093 \\ 0.034 \\ 0.110 \\ 0.151 \\ 0.261 \\ 0.206 \\ 0.467 \end{array} $	$ \begin{vmatrix} 0.414 \\ 0.322 \\ 0.092 \\ 0.026 \\ 0.126 \\ 0.222 \\ 0.348 \\ 0.177 \\ 0.525 \\ \end{vmatrix} $	0.403 0.308 0.095 0.025 0.145 0.195 0.340 0.339 0.679	
			Stems							
Total assimilated N. Protein N. Soluble organic N. Nitrate N. Reducing sugars Sucrose. Total sugars. Starch + dextrins Total carbohydrate.	0.181 0.099 0.081 0.077 0.122 0.252 0.373 0.268 0.641	$\begin{array}{c} 0.194 \\ 0.120 \\ 0.074 \\ 0.059 \\ 0.116 \\ 0.260 \\ 0.375 \\ 0.168 \\ 0.544 \end{array}$	$\begin{array}{c} 0.206 \\ 0.126 \\ 0.080 \\ 0.060 \\ 0.111 \\ 0.298 \\ 0.409 \\ 0.259 \\ 0.668 \end{array}$	0.198 0.111 0.088 0.060 0.153 0.324 0.477 0.376 0.853	0.197 0.109 0.087 0.062 0.155 0.362 0.518 0.370 0.888	0.213 0.133 0.080 0.061 0.149 0.264 0.413 0.238 0.651	0.209 0.135 0.074 0.058 0.163 0.250 0.413 0.246 0.659		$ \begin{array}{c} 0.200 \\ 0.128 \\ 0.072 \\ 0.058 \\ 0.203 \\ 0.239 \\ 0.442 \\ 0.316 \\ 0.758 \end{array} $	
			Roots							
Total assimilated N. Protein N. Soluble organic N. Nitrate N. Reducing sugars. Sucrose. Total sugars. Starch + dextrins. Total carbohydrate.	0.154 0.120 0.033 0.060 0.033 0.065 0.098 0.005 0.102	$\begin{array}{c} 0.148 \\ 0.116 \\ 0.032 \\ 0.044 \\ 0.037 \\ 0.069 \\ 0.102 \\ 0.027 \\ 0.132 \\ \end{array}$	$\begin{array}{c} 0.134 \\ 0.101 \\ 0.032 \\ 0.049 \\ 0.036 \\ 0.060 \\ 0.096 \\ 0.030 \\ 0.126 \end{array}$	$\begin{array}{c} 0.126 \\ 0.096 \\ 0.030 \\ 0.043 \\ 0.041 \\ 0.131 \\ 0.172 \\ 0.040 \\ 0.212 \end{array}$	0.128 0.098 0.030 0.047 0.040 0.116 0.157 0.026 0.182	0.160 0.134 0.027 0.049 0.036 0.065 0.101 0.002 0.103	$\begin{array}{c} 0.176 \\ 0.150 \\ 0.027 \\ 0.046 \\ 0.041 \\ 0.078 \\ 0.119 \\ 0.023 \\ 0.142 \end{array}$	0.167 0.144 0.023 0.044 0.040 0.093 0.133 0.024 0.157	0.166 0.140 0.025 0.042 0.052 0.092 0.144 0.027 0.171	

percentage of total nitrogen present in the various portions of the plants, and that the relative amount of nitrogen in the calcium chloride plants was appreciably less than in the sodium chloride plants. Data in Table II show that the amount of unassimilated nitrogen, which had accumulated in these plants prior to utilization, was definitely decreased by the presence of added salt in the substrate. The leaves showed a continual diminution of nitrate content with increasing osmotic concentrations. Although the stems and roots contained less nitrate in the presence of any amount of added salt in nutrient solutions than those from the base nutrient treatment, the salt concentration had practically no effect on nitrate percentages. When these results are interpreted in view of the fact that growth was markedly inhibited by salt increments, it is evident that the absolute amount of nitrate absorbed by these plants had been considerably decreased by the presence of the salts. Although the amount of nitrate in a plant is an index of the reserve of unassimilated nitrogen available for metabolic processes, the soluble organic component serves as a more valid index of the status of nitrogen metabolism. Data in Table II show that there were few marked trends in the soluble organic nitrogen fraction for any of the parts of these plants as conditioned by osmotic concentration of the growing medium. Yet, in each tissue, there appears a consistently

higher proportion of soluble organic nitrogen in the plants of sodium chloride series than in those of the calcium chloride series. It is true that the differences were not large, but their consistency tends to enhance their validity. It is possible that these differences are related to the tendency of the calcium ion to be less conducive to hydration than the sodium ion, accounting for the lower proportion of simpler nitrogenous compounds in the calcium plants. This is shown in Table III.

TABLE III-PROTEIN AND SOLUBLE ORGANIC NITROGEN IN BEAN PLANTS AS PROPORTIONS OF TOTAL ASSIMILATED NITROGEN

Base Nutrient (Per Cent) NaCl Series (Per Cent) CaCl. Series (Per C											
Osmotic Concent	ration	0.5	1.5	2.5	3.5	1.5 1.5	2.5	3.5	4.5		
			Leave	s							
Protein as per cent of total assimilated N.	80.3	76.8	77.3	76.3	76.8	81.4	78.8	77.8	76.4		
Soluble organic as per cent of total assimilated N	19.7	23.2	22.7	23.7	23.2	19.6	21.2	22.2	23.6		
			Stems								
$\begin{array}{c} \textbf{Protein as per cent of total} \\ \textbf{assimilated}  N \dots \end{array}$	54.7	61.9	61.2	56.1	55.3	62.4	64.6		64.0		
Soluble organic as per cent of total assimilated N.	44.8	38.1	38.8	43.9	44.7	37.6	35.4	_	36.0		
			Roots								
Protein as per cent of total assimilated N	77.9	78.4	75.4	76.2	76.6	83.8	85.2	86.2	84.3		
Soluble organic as per cent of total assimilated N	21.1	21.6	24.6	23.8	23.4	16.2	14.8	13.8	15.7		

One might expect that increasing hydrostatic stress would be conducive to condensation of simple nitrogenous compounds to complex proteins. Nightingale and Farnham (4) observed this to be the case. The present data show that the amount of protein in the leaves, which are the main reservoir of protein in these plants, was decreased by successive increments of salt in the substrate. It is to be recalled that Nightingale and Farnham (4) increased osmotic concentration by uniformly increasing the concentration of all salts in the base nutrient solution so that nitrogen supply and relative absorption was greatly increased in their plants. The added salts not only inhibited nitrogen absorption in the present study, but also the amount of protein in the leaves, both as percentage composition of the leaves and as a proportion of the total assimilated nitrogen therein. In the stems and roots it is pertinent that the assimilated nitrogen contained a higher proportion of protein nitrogen within the calcium chloride plants than in the sodium chloride plants. And, accordingly, the soluble organic nitrogen relationship was just the reverse. The specific effect of the calcium and sodium ions on colloidal systems coincides with these observed shifts in metabolic constituents. It should be stressed that this effect was largely in the roots, and that the growth data showing differentials between the calcium and sodium series of plants are

undoubtedly largely related to this differential in the metabolic status of the roots.

The data for the carbohydrate fractions in the plants emphasized mainly that the aerial environment under which these plants were grown caused plants in all treatments to be relatively very low in all carbohydrate components, especially starch. There was a general tendency for sugar to accumulate with increasing osmotic concentration of the substrate; whereas, the observations on starch were rather erratic, showing no accumulation with added salt, but actually a marked decrease in the amount of starch in the plants at the low salt concentrations. Such accumulation of sugars may well have been anticipated. They would be largely responsible for an increase in the osmotic value of the tissues of these plants; for such an increase may have been reasonably expected to accrue from an increase in osmotic concentration of the substrate.

## SUMMARY

Growth of red kidney bean plants as measured by green weight showed a linear decrease with increasing increments in osmotic concentration of the substrate. Plants in the calcium chloride series made consistently less growth than in the sodium chloride series and the difference was largely due to poorer root growth in the calcium series. Consequently the top root ratios were appreciably higher in the latter series.

Increments of added salts in the substrate were associated with decreases in the percentage of nitrate nitrogen in the plants. Salt concentrations per se did not modify the percentage of soluble nitrogen in the plants, but the values were consistently higher in the sodium chloride series than in the calcium chloride series of plants. Percentage of protein diminished with increments of added salt. Protein nitrogen constituted a much higher proportion of the assimilated nitrogen in the stems and roots of the calcium chloride series of plants. It was suggested that the differential effects of Na+ and Ca++ ions on hydration of colloids may be involved and the results obtained are in complete agreement with such known effects.

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